ASCOGENOUS FORMS OF GLOEOSPORIUM AND COLLETOTRICHUM

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An accurate knowledge of the life-histories of fungi is of the greatest importance, not only to taxonomy, but especially to plant pathology. As many parasitic fungi possess two or more fruiting stages, in some cases occurring on different hosts, it is necessary to have a full knowledge of their complete course of development in order to know how they are reproduced and distributed, and to devise adequate methods of combating or preventing them.

The fungi producing the diseases known as anthracnoses, which attack a great variety of wild and cultivated plants, have been described chiefly as species of Gloeosporium and Colletotrichum. The positive connection of these conidial forms with ascogenous stages was, so far as we are aware, first demonstrated by means of pure cultures by Professor G. F. ATKINSON, as reported by Miss STONEMAN[1] in 1898. FÜCKEL[2] in 1869 gave *Gloeosporium juglandis* Lib. (*Marsonia juglandis* (Lib.) Sacc.) as the spermagonial form of *Gnomonia leptostyla* (Fr.) Ces. & De Not., which occurs on *Juglans regia*. It has uniseptate conidia, and the ascospores are also uniseptate, which facts seem to indicate that it is not congeneric with the organisms with which we are concerned at present. FÜCKEL'S statement in regard to the connection between these two forms was based simply upon the association of the two on the same leaf, and was merely a guess. KLEBAHN[3], however, has recently demonstrated by pure cultures and infection experiments that FÜCKEL was correct in this case. Other forms studied by KLEBAHN, which have passed under the name of Gloeosporium, are *G. nervisequum* (Fück.)

[1] STONEMAN, B., The development of some anthracnoses. BOT. GAZETTE **26**: 69. 1898.

[2] FÜCKEL, L., Symbolae Mycologicae 123. 1869.

[3] KLEBAHN, H., Zuzammenhänge von Ascomyceten mit Fungis imperfectis. Cent. f. Bakt. **15**[2]:336. 1905.

Sacc.[4] and *G. Ribis* (Lib.) Mon. & Desm.[5] The first of these he connects with *Gnomonia veneta* (Sacc. & Speg.) Kleb., which has uniseptate ascospores, and hence is not congeneric with the anthracnoses, whose ascospores are not septate. In the case of *G. Ribis*, KLEBAHN[5] claims to have demonstrated by infection experiments and by pure cultures that its ascogenous stage is a discomycete, which he has described as *Pseudopeziza Ribis*. It is very remarkable if conidial forms so similar as to be placed by taxonomists in the same genus should have ascogenous stages belonging to such different, distinct groups as the Pyrenomycetes and the Discomycetes. Miss STONEMAN (*l.c.*) in 1898, CLINTON[6] in 1902, VON SCHRENK and SPAULDING[7] in 1903, SHELDON[8] in 1905 and 1906, and SCOTT[9] in 1906 have worked with organisms from various hosts which have usually passed under the name of Gloeosporium or Colletotrichum, and which are undoubtedly congeneric with the plants treated of in this paper.

We may say, in the beginning, that neither of the names just mentioned is tenable for any of the plants to be considered here, as these names were originally applied to species belonging to other genera than those to which they are applied by SACCARDO and other recent mycologists. The name Glomerella, of SPAULDING and VON SCHRENK, will be used here, as it is the only one which we are absolutely certain at present belongs to this group of organisms. There is little doubt, however, that there are older valid generic names which have been applied to either the conidial or ascogenous stages of these

[4] KLEBAHN, H., Untersuchungen über einige Fungi imperfecti und die zugehörigen Ascomycetenformen. II. Jahrb. Wiss. Bot. **41**:515. 1905.

[5] ———, Untersuchungen über einige Fungi imperfecti und die zugehörigen Ascomycetenformen. III. Zeits. Pflanzenkrank. **16**:65. 1906.

[6] CLINTON, G. P., Apple rots in Illinois. Agr. Exp. Sta. Bull. no. 69. 1902.

[7] SCHRENK, H. VON, and SPAULDING, P., The bitter-rot of the apple. U. S. Dept. Agr., Bur. Pl. Ind., Bull. no. 44. 1903.

[8] SHELDON, J. L., Concerning the identity of the fungi causing an anthracnose of the sweet-pea and the bitter-rot of the apple. Science N. S. **22**:51. 1905.

———, The ascigerous stage of *Gloeosporium Psidii*. Science N. S. **21**: 143. 1905.

———, The ripe rot or mummy disease of guavas. W. Va. Agr. Ex. Sta. Bull. no. 104. 1906.

[9] SCOTT, W. M., The control of the apple bitter-rot. U. S. Dept. Agr., Bur. Pl. Ind., Bull. no. 93. 1906.

fungi, but until we have had opportunity to study the types of the genera to which they may belong, we prefer to use the name Glomerella for at least all the species which are known to have ascogenous stages congeneric with *Glomerella rufomaculans* (Berk.) Spauld. & v. Schrenk.

The first published work on the life-history of these fungi was that of Miss STONEMAN, in 1898; she worked with twelve forms from as many different hosts, and succeeded in producing in pure cultures both the conidial and ascogenous stages in four cases, and a doubtful fifth, as follows:

Glomerella cingulata (Stoneman) Spauld. & v. Schrenk, the conidial stage occurring on privet (*Ligustrum vulgare*); *G. piperata* (Stoneman) Spauld. & v. Schrenk, the conidial stage found on pepper, *Capsicum* sp.; *G. cincta* (Stoneman) Spauld. & v. Schrenk, the conidial stage occurring on an orchid, *Maxillaria picta*, and provisionally referred to Colletotrichum; *G. rubicola* (Stoneman) Spauld. & v. Schrenk, the conidia from red raspberry, *Rubus strigosus;* *G.* (?) *Vanillae* (Stoneman) Spauld. & v. Schrenk, the conidia from the cultivated vanilla.

Miss STONEMAN referred all these species to a new genus, which she named *Gnomoniopsis*. This name was untenable, however, having been previously used by BERLESE[10] for another genus of fungi. CLINTON (*l. c.*) in 1902, seems to have been the first to obtain in pure cultures the ascogenous stage of the Gloeosporium causing anthracnose or bitter-rot of the apple. VON SCHRENK and SPAULDING (*l. c.*) in 1903, and SCOTT (*l. c.*) in 1906, also worked with the apple Gloeosporium and made additions to our knowledge of its life-history and conditions of development. SHELDON (*l. c.*) in 1906, reported the successful growth in pure cultures of the ascogenous stage of the guava anthracnose or ripe-rot, *Glomerella Psidii* (Del.) Sheldon. These six forms are the only ones whose life-histories have heretofore been determined by pure culture methods, so far as we have been able to learn from published records.

The present writers have studied a number of forms and have succeeded in growing both the conidial and ascogenous stages from eight different hosts, as follows:

Gloeosporium rufomaculans (Berk.) v. Thümen, from the cultivated grape, *Vitis* sp.; *G. fructigenum* Berk., from the apple; an apparently unnamed Gloe-

[10] BERLESE, A. N., Icones Fungorum 1:93. 1892.

osporium from the cranberry, *Vaccinium macrocarpum;* *G. elasticae* Cooke & Massee, from the leaves of the rubber plant, *Ficus elastica;* a form from the locust, *Gleditschia triacanthus*, which does not appear to have been reported before; one from *Ginkgo biloba*, also not heretofore reported; *Colletotrichum gossypii* Southw., from cotton; and *C. Lindemuthianum* (Sacc. & Magnus) Bri. & Cav., from the cultivated bean.

Of these eight only the one from the apple has had its ascogenous form reported heretofore. Miss STONEMAN worked with the anthracnose of the bean, but was unable to produce the ascogenous stage.

All the forms mentioned have been grown by the writers in pure cultures, and the ascogenous stages produced, in most cases, several times. The original cultures were usually obtained by the transfer to culture media of conidia or portions of the tissue of the host containing the hyphae of the fungus. We have been unable as yet to tell definitely what the factors are which control the production of the ascogenous form. Repeated experiments with various culture media, under various conditions of light, temperature, and moisture, seem to indicate that these are not factors of great importance. Once having obtained a race, strain, or generation which produces ascogenous perithecia in cultures, it can be successfully grown on various media and under various conditions for several generations.

The medium which we have found best adapted for use and which has produced the most abundant and luxuriant growths of ascogenous forms is sterilized corn meal. We have found by repeated experiments that if a culture from any particular acervulus or group of acervuli does not produce an ascogenous stage on corn meal at 75° to 85° F., it is useless to experiment further with material from the same source. Fresh material from other specimens must be tried until a race, strain, or generation is found which will produce its ascogenous form. It has been suggested that the nearness or remoteness of origin of the conidia from ascospores may be an important factor in determining the production of an ascogenous stage. The few experiments we have made along this line are not conclusive, and more data must be obtained before results can be reported.

Heretofore forms occurring on different hosts have been generally regarded as distinct species. The study which we have made of the eight forms just mentioned leads to the conclusion that they cannot

be successfully segregated as species upon the basis of morphological characters. Though we have examined a great deal of material, of both conidial and ascogenous stages, from various pure cultures, as well as from the natural hosts, we are unable to find any differences of sufficient constancy or importance to make it possible to distinguish species or even varieties except the host be considered. Miss STONEMAN describes and figures most of her species as having perithecia with a distinct neck or beak. We have very rarely seen in any of our cultures as conspicuous a beak as she figures. In all other characters, however, the forms we have studied agree with hers. The conidia of these fungi are well known to be quite variable in size and shape, and it is frequently possible to find in a single acervulus conidia varying in length from 10 to 25 μ and in thickness from 3.5 to 6 μ, so that the range of variation in a single culture will usually cover the range of variation found in any of the forms we have studied. This is not only true of the conidia, but is also true of the ascogenous forms. The greatest variation found in the size of ascospores is from 9 to 24 μ by 4.5 to 8 μ, the usual measurements being about 14 to 18 μ by 5 to 6 μ. In shape the asci vary somewhat, being usually sub-cylindrical or clavate. The ascospores vary but little in shape and appearance of contents. They are usually slightly curved or allantoid, having the contents in fresh specimens regularly granular, with a more or less distinct hyaline zone at the center, and when fully matured are of a light greenish-yellow color. This color, however, is hardly noticeable except in mature specimens. From this it will be seen that these organisms cannot be satisfactorily distinguished by their morphological characters, so far as the conidia and asci are concerned. Some of the conidial forms have been separated heretofore upon the basis of the presence or abscence of dark setae in the acervulus. This, as has been pointed out by other writers, is also an uncertain and variable character. We have found setae in the acervuli in one part of a pure culture, whereas in other parts of the same culture they were absent. We have also found this same variation in acervuli occurring upon leaves, especially in the form found upon the cranberry.

The question remains whether these organisms can or should be separated as physiological species. The answer depends upon

whether the forms are restricted to their individual hosts, or can be successfully transferred from one host to another. The work already done by HALSTED,[11] SOUTHWORTH,[12] and others in transferring various forms occurring upon different fruits from one host to another by inserting the conidia in the tissue of the host, or by applying them to the surface of mature fruits does not seem to us to be conclusive. A mature fruit, especially when the surface is ruptured, might be regarded simply as a culture medium, and the fact that these organisms will grow upon it does not prove that they would grow upon the plant as a parasite. The only conclusive test of this matter must be made by careful infection experiments, using fresh, living, and growing plants controlled by checks. SHELDON[13] reports having done this in the case of the anthracnose of the apple, having succeeded in infecting the leaves of the sweet-pea by transferring conidia from the apple. These experiments, in connection with others which have been reported to us verbally, seem to indicate that some of these forms are not physiologically distinct. In some cases at least the evidence indicates that plants which appear free from disease are already infected, the fungus apparently being in a dormant or more or less inactive condition and awaiting favorable conditions for development. In the anthracnose of cotton and bean, the disease is known to be transmitted by diseased seed. It is desirable, therefore, that plants used for infection experiments should be grown from healthy disinfected seed under such conditions as to prevent the possibility of infection from other sources. In the present state of our knowledge, perhaps it may be best to regard the various forms we have studied as varieties of one species.

The bodies frequently found in nature and in cultures, which have been called by some chlamydospores and by others appressoria, show no specific characters. While their function, perhaps, may be primarily that of appressoria or hold-fast organs, as maintained by HASSELBRING[14] and others, they are often produced under conditions

[11] HALSTED, B. D., Laboratory study of fruit decays. Report N. J. Agr. Exp. Sta. **1892**: 326. 1893.

[12] SOUTHWORTH, E. A., Ripe rot of grapes and apples. Jour. Myc. **6**: 164. 1891.

[13] SHELDON, J. L., Concerning the identity of the fungi causing an anthracnose of the sweet-pea and the bitter-rot of the apple. Science N. S. **22**: 51. 1905.

[14] HASSELBRING, H., Appressoria of the anthracnoses. BOT. GAZETTE **42**: 135. 1906.

which seem to indicate that they may have some other function as well. They have frequently been found in our cultures, and occur also in cranberries that have been destroyed by the cranberry anthracnose. They vary considerably in size and shape, and the germ pore which is attributed to them is frequently indistinct or wanting.

Most writers who have studied the ascogenous stages of these fungi have described them as without paraphyses. SHELDON[15] has recently mentioned finding paraphyses in ascogenous perithecia produced from the apple. We have occasionally found organs surrounding the outer portion of the mass of asci which bear a close resemblance to the paraphyses of certain other pyrenomycetous fungi. A careful study of these organs shows that they are not intermingled with the asci, but are about the outer portion of the mass, next to the wall of the perithecium. In many instances they suggest aborted or malformed asci. In any case they would be more correctly called periphyses than paraphyses. Their rare occurrence would seem to indicate that they are of no great importance for taxonomic purposes.

Since these organisms have been found to produce ascogenous perithecia in cultures, it would be expected that they would also produce them under natural conditions upon their host plants. The conidial forms are so numerous and so widely distributed that if the ascogenous forms occur often they would probably have been found and described by mycologists before now. DELACROIX[16] describes *Glomerella* (?) *Artocarpi* as found on Artocarpus leaves associated with Gloeosporium and Colletotrichum. The description and figures agree well with our ascogenous forms. Since beginning this investigation we have examined carefully various plants attacked by anthracnose in the hope of finding ascogenous perithecia under natural conditions. Thus far, however, we have been able to find them in only two cases; that of the apple, which had already been reported by CLINTON (*l. c.*), and that of the rubber plant, *Ficus elastica*. The ascogenous perithecia are frequently found in abundance upon fallen leaves of the rubber plant, which have been attacked by the anthracnose, *Gloeosporium elasticae* Cke. & Mass. They agree in

[15] SHELDON, J. L., The ripe rot or mummy disease of guavas. W. Va., Agr. Exp. Sta., Bull. 104: 310. 1906.

[16] DELACROIX, G., Bull. Soc. Myc. France **21**: 198. 1905.

every respect with those which are produced in pure cultures, except that the perithecia in the pure cultures are more or less overgrown with the vegetative hyphae, and this we find to be generally true in the case of cultures of perithecia of other fungi, which under natural conditions are borne within the tissues of the host. There are several genera of pyrenomycetous fungi which have characters so nearly like those of Glomerella that they cannot be very satisfactorily distinguished from it, and we are of the opinion that some of the species already referred to these genera are really the ascogenous stages of anthracnoses. Of such genera we may mention Physalospora, Phomatospora, and Guignardia. As already stated, for the present it seems advisable to treat these organisms as varieties of the oldest species, *Glomerella rufomaculans* (Berk.) Spauld. & v. Schrenk, which was originally described from specimens from the grape.

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